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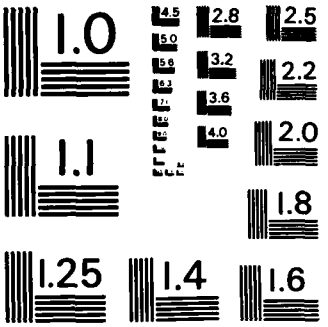

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SEQUENCE POLYMORPHISM OF HLA-DRβ1 ALLELES RELATING TO T CELL-RECOGNIZED DETERMINANTS

J.S. Cairns, J.M. Curtsinger, C.A. Dahl,

S. Freeman, B.J. Alter, and F.H. Bach.

Immunobiology Research Center

Departments of Laboratory Medicine/Pathology and Surgery

Box 724 Mayo Building

University of Minnesota

Minneapolis, Minnesota 55455.

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INTRODUCTION

HLA class II molecules are a highly polymorphic family of dimeric cell surface proteins primarily involved in regulating T cell responses to extrinsic antigens. To define regions of class II molecules involved in T cell recognition, we have compared sequences of three HLA-DRβ cDNA clones obtained from cells that all express the same serologically defined determinants but differ in terms of T cell-recognized specificities. The comparisons indicate that very few (one to four) nucleotides differ between what are almost certainly alleles of the DRβ1 locus. All differences were in the first domain of the molecule and all localized to a region from amino acids 71-86. Since all differences were found only in this region of the molecule, and since DR α chains seem to be relatively nonpolymorphic[1], these positions in the DRβ chain must play a major role in influencing T cell recognition of the DR molecule.

Serological and molecular cloning experiments have shown that there are three families of class II molecules within the HLA complex, termed DP, DQ, and DR. For the DR4-DRw53 haplotype which we have studied, within the DR family, one α and three complete β genes are known; one β gene is a pseudogene[2]. At least two DR αβ dimers are expressed. The serologic determinant DR4 is associated with the αβ1 dimer whereas the DRw53 determinant is associated with the αβ2 dimer. To date, comparisons of DRβ nucleic acid sequences have been made between cells differing both serologically and in terms of T lymphocyte recognized determinants, making it impossible to ascribe determinants recognized by T lymphocytes to particular polymorphic sequences. To minimize differences to those potentially involved in T cell recognition, we have compared sequences of what appear to be DRβ1 cDNA clones from cells which differ with respect to determinants, designated as "Dw", recognized by

allogeneic T cells, but share the serologically-defined specificity DR4.

Among cells which express DR4, at least 5 distinct Dw subtypes have been defined, designated Dw4, Dw10, Dw13, Dw14, and Dw15.[3] Clones LS5.8.1 and S3.4 were obtained from cDNA libraries of two homozygous typing cells (HTCs), LS40 and SSTO, expressing Dw14 and Dw13 respectively. The sequences of these clones are shown in Fig. 1. These clones differ by a single nucleotide resulting in an alanine in LS5.8.1 (Dw14) and a glutamic acid in S3.4 (Dw13) at amino acid position 74 (table 1, lines a and b).

LS5.8.1 differs by only three nucleotides (two amino acids, table 1, lines a & c) from an unpublished DRβ sequence (Long, Mach et al., personal communication), from a DR4+ cell, referred to as "DR4,6". To make this sequence comparison more meaningful, we used cloned cytotoxic T lymphocytes that differentiate between the DR products (most likely DRβ1)[4] of Dw4 and Dw14, and established that DR components of Dw4 are associated with the DR4 haplotype of "DR4,6". As shown in table 2, both LS40 and "DR4,6" served as effective targets for cloned CTL which recognize both Dw4 and Dw14 associated determinants; only "DR4,6" was lysed by cloned CTL recognizing Dw4 but not Dw14 determinants.

Based on the high degree of sequence homology between the DRβ clones from Dw14, Dw13, and Dw4 cells described, it is extremely likely that these clones represent transcripts of alleles of a single locus. These clones presumably represent transcripts of the DRβ1 locus for the following reasons. Speis, Strominger et al.[5] have compared sequences of the first domains of known (based on amino acid sequencing of characterized proteins) DRβ1 and DRβ2 genomic clones from Priess (a DR4-Dw4 HTC) and have found 19 amino acid differences between them. The DR4-Dw4 β1 sequence from Priess is identical to the sequence of "DR4,6".

On allelic sequences, because they differ by 2 to 3 amino acids from the DRβ1 sequence of Priess, but by 16 to 17 amino acids from the DRβ2 sequence, most likely represent DRβ1 sequences. Also apparent from this comparison is that at the positions where the DRβ1 sequences of Dw14, Dw13, and Dw4 differ from each other, one of the alternative amino acids and the corresponding codon is also present at the same position in the DRβ2 sequence. Whether this has occurred as the result of accumulated mutation in the DRβ1 gene, or as a result of a gene conversion-like mechanism, is not clear.

The conclusion that these sequences represent DRβ1 transcripts is also consistent with the observation that there is an approximately ten-fold higher level of DRβ1 as compared with DRβ2 expressed on the surface of EBV-transformed B cells[5,6]; that finding may well be reflected in levels in the mRNA pool. We have now sequenced all or part of three different DRβ cDNA clones from our LS40 library and, although there is evidence to suggest that transcripts of this gene are differentially processed (Cairns, S., Dahl, C., Curtsinger, C., and Bach, F.H., in preparation) all are apparently transcripts of a single locus. The clone sequenced by Long et al. also is a member of a highly represented family of DRβ clones (group 2 in reference 7).

Based on the likelihood that these sequences represent transcripts of the DRβ1 locus, it is apparent from these data that a few amino acid differences may profoundly affect how the molecule is recognized by T lymphocytes. This situation is not without precedent; relatively minor amino acid substitutions in the heavy chain of the HLA-A2 molecule[8,9] and in mutants of the K^b molecule[10] drastically alter T cell recognition of those molecules.

Structural models of the DR molecule have placed residues 71, 74, and 86 on the outer face of the DR molecule[11]. Residues 71 and 74 are included in the third hypervariable region of the DRβ chain, the only proposed α-helical

region in the first domain[11]. Amino acid substitutions in the I-A mutant, bm12 are in this same region[12]. To the extent that determinants of the DRβ1 dimer are involved in restricted recognition of foreign (nominal) antigens, for which there is some evidence[13,14], these same amino acid differences among alleles of DR β1 must be involved.

Whether the changes in DNA sequence noted in this paper are directly responsible for encoding T cell recognized determinants or whether they are responsible for conformational changes involving other parts of the molecule which are recognized by T cells is not addressed by this approach. We would expect, in addition, to find other sequence differences that could result in determinants recognized by T cells. We would postulate, however, as we have recently discussed[15,16], that the allelic differences reported here in a naturally evolving population may be of primary importance evolutionarily and functionally for T lymphocyte recognition.

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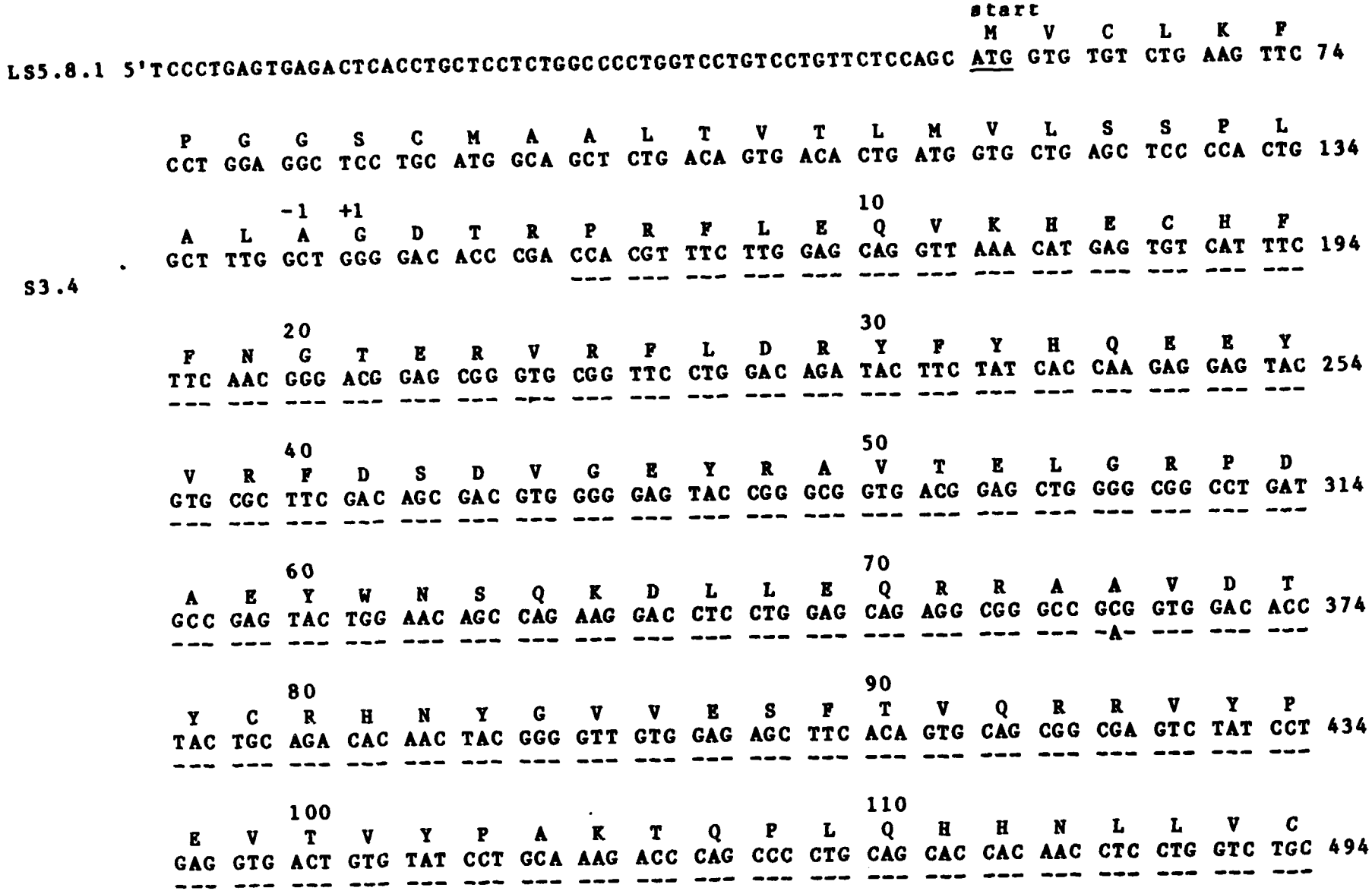
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M V C L K F
LS5.8.1 5'TCCCTGAGTGAGACTCACCTGCTCCTCTGGCCCCTGGTCCTGTCCTGTTCTCCAGC ATG GTG TGT CTG AAG TTC 74
P G G S C M A A L T V T L M V L S S P L
CCT GGA GGC TCC TGC ATG GCA GCT CTG ACA GTG ACA CTG ATG GTG CTG AGC TCC CCA CTG 134
-1 +1 10
A L A G D T R P R F L E Q V K H E C H F
GCT TTG GCT GGG GAC ACC CGA CCA CGT TTC TTG GAG CAG GTT AAA CAT GAG TGT CAT TTC 194
S3.4
20 30
F N G T E R V R F L D R Y F Y H Q E E Y
TTC AAC GGG ACG GAG CGG GTG CGG TTC CTG GAC AGA TAC TTC TAT CAC CAA GAG GAG TAC 254
40 50
V R F D S D V G E Y R A V T E L G R P D
GTG CGC TTC GAC AGC GAC GTG GGG GAG TAC CGG GCG GTG ACG GAG CTG GGG CGG CCT GAT 314
60 70
A E Y W N S Q K D L L E Q R R A A V D T
GCC GAG TAC TGG AAC AGC CAG AAG GAC CTC CTG GAG CAG AGG CGG GCC GCG GTG GAC ACC 374
80 90
Y C R H N Y G V V E S F T V Q R R V Y P
TAC TGC AGA CAC AAC TAC GGG GTT GTG GAG AGC TTC ACA GTG CAG CGG CGA GTC TAT CCT 434
100 110
E V T V Y P A K T Q P L Q H H N L L V C
GAG GTG ACT GTG TAT CCT GCA AAG ACC CAG CCC CTG CAG CAC CAC AAC CTC CTG GTC TGC 494

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          120                                                 130
 S    V    N    G    F    Y    P    G    S    I    E    V    R    T    F    R    N    G    Q    E
TCT  GTG  AAT  GGT  TTC  TAT  CCA  GGC  AGC  ATT  GAA  GTC  AGG  TGG  TTC  CGG  AAC  GGC  CAG  GAA 554
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          140                                                 150
 E    K    T    G    V    V    S    T    G    L    I    Q    N    G    D    W    T    F    Q    T
GAG  AAG  ACT  GGG  GTG  GTG  TCC  ACA  GGC  CTG  ATC  CAG  AAT  GGA  GAC  TGG  ACC  TTC  CAG  ACC 614
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          160                                                 170
 L    V    M    L    E    T    V    P    R    S    G    E    V    Y    T    C    Q    V    E    H
CTG  GTG  ATG  CTG  GAA  ACA  GTT  CCT  CGG  AGT  GGA  GAG  GTT  TAC  ACC  TGC  CAA  GTG  GAG  CAC 674
---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  --N

          180                                                 190
 P    S    L    T    S    P    L    T    V    E    W    R    A    R    S    E    S    A    Q    S
CCA  AGC  CTG  ACG  AGC  CCT  CTC  ACA  GTG  GAA  TGG  AGA  GCA  CGG  TCT  GAA  TCT  GCA  CAG  AGC 734
NNN  NNN  NNN  NNN  NN-  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---

          200                                                 210
 K    M    L    S    G    V    G    G    F    V    L    G    L    L    F    L    G    A    G    L
AAG  ATG  CTG  AGT  GGA  GTC  GGG  GGC  TTC  GTG  CTG  GGC  CTG  CTC  TTC  CTT  GGG  GCC  GGG  CTG 794
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          220                                                 230
 F    I    Y    F    R    N    Q    K    G    H    S    G    L    Q    P    T    G    F    L    S
TTC  ATC  TAC  TTC  AGG  AAT  CAG  AAA  GGA  CAC  TCT  GGA  CTT  CAG  CCA  ACA  GGA  TTC  CTG  AGC 854

stop
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ATTCTTCCACAAGAGAGACCTTTCTCCGGACCTGGTTGCTACTGGTTCAGCAGCTCTGCAGAAAATGTCCTCCCTTGTG 1012

GCTGCCTCAGCTCGTACCTTTGGCCTGAAGTCCCAGCATTAATGGCAGCCCCTCATCTTCCAAGTTTTGTGCTCCCCTT 1091

TACCTAATGCTTCCTGCCTCCCATGCATCTGTACTCCTGCTGTGCCACAAACANATTACATTATTAAATGTTTCTCAAA 1170

CATGGAGTTAA 3'
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Legend to Figure 1.

Both LS5.8.1 and S3.4 were initially isolated from cDNA libraries constructed from membrane-bound (LS5.8.1) or cytoplasmic (S3.4) RNA. cDNA was synthesized using oligo-dT to prime the first strand, and nascent mRNA nicked with RNAse H to prime the second strand. cDNA was "blunt-ended" with DNA polymerase and tailed with dCTP using terminal deoxynucleotidyltransferase. cDNA was annealed with Pst 1-cut, dG-tailed pUC9, and introduced into E. coli stain 83. Each library, which contained approximately 7000 colonies was screened with a 790 bp Sst1-Hind111 fragment of a DRβ cDNA clone isolated by E. Long et al.[7]. This probe contains nearly the entire coding region from this clone and under the conditions or hybridization employed, reacts most strongly with DRβ cDNA but also reacts weakly with DPβ and DQβ. The inserts from these clones, or fragments thereof, were subcloned into the single-stranded vector M13mp19. Subclones were either sequenced directly or truncated subclones were generated by the method of Dale et al.[17]. Sequencing was done using the dideoxy chain termination method[18]. "N's" in the S3.4 sequence represent unsequenced areas of the insert. In addition, approximately 110 nucleotides at the 5' end of this insert have not yet been sequenced. Where the sequence of S3.4 is identical to that of LS5.8.1, a dash is shown below the LS5.8.1 sequence.

Table 1. Summary of amino acid differences between DRβ1 alleles.

	clone	cell	DR	Dw	amino acid position 71	74
a.	LS5.8.1	LS40	β1	14	Arg	Ala
b.	S3.4	SSTO	β1	13	Arg	Glu
c.		"DR4,6"[1]	β1	4	Lys	Ala
d.		Preiss[2]	β1	4	Lys	Ala
e.		Preiss[2]	β2	4	Arg	Glu

[1]Taken from the unpublished sequence of a DR β cDNA clone obtained by E. Long, B. Mach and coworkers.

[2]Taken from T. Speiss et al.[5].

Table 2. Lysis of "DR4,6", LS40 and other targets by cytotoxic T lymphocyte clones.

	Target				
	"DR4/6"	LS40	BO (Dw4 HTC)	K.Blo (Dw3,4)	J.Nor (Dw2,3)
clone[x]					
KD15	43.0 ± 4.4[†]	0.8 ± 1.4	35.7 ± 4.6	40.5 ± 6.3	2.1 ± 1.3
KD33	59.2 ± 6.6	2.1 ± 1.2	27.2 ± 3.9	40.0 ± 2.8	10.1 ± 2.0
KD48	55.4 ± 8.3	56.3 ± 9.5	16.1 ± 2.7	23.4 ± 2.7	1.1 ± 2.5

[x] KD15 and KD33 are Dw4 specific; KD48 lyses Dw4 and Dw14 targets[4].

[†] % CML ± S.D. The effector:target ratio is 20:1.

Legend to Table 2

The cell-mediated lympholysis (CML) assay was performed as follows. Target cells were labeled with 0.25 mCi chromium 51 for 1 hour, washed 3 times with cold RPMI-1640 containing 20% pooled human serum, and adjusted to 1 x 10^4 cells/ml. The cloned effectors were resuspended in IL-2-free culture medium the day prior to testing. Effectors were adjusted to the appropriate concentration and 100µl effectors + 100µl targets were added to V bottom microtiter plates, spun at 500 rpm for 5 minutes and incubated in a 37°, 5% CO_2 humidified environment for 4 hours. The plates were then spun at 1000 rpm for 10 minutes and 150µl of supernatant were aspirated and placed in scintillation vials. Ready-Solv™ HP (Beckman 566436) (2.5 ml.) was added to each vial and the samples were counted in a β scintillation counter. Spontaneous release of ^{51}Cr was assessed by incubating target cells without effector cells and maximum release of the isotope was assessed by incubating target cells with 0.1% hexadecyltrimethylammonium bromide. Results were calculated as follows:

$$\text{percent cytotoxicity} = \frac{\text{cpm experimental wells} - \text{cpm spontaneous release}}{\text{cpm maximum release} - \text{cpm spontaneous release}} \times 100$$

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